Product Information Sheet

Micro BCA Protein Assay Kit

Catalog No. AR1110

Kit Contents

Micro BCA Reagent A (MA) 100 ml

Micro BCA Reagent B (MB) 100 ml

Micro BCA Reagent C (MC) 5 ml

Diluted Albumin (BSA) Standards Ampules

(10mg/vial） 5 vials

Storage

Store at 4℃ (BSA Albumin Standard Ampules should be stored at -20℃)

Expiration Date

At least one year

Tested assay

Tube procedure: 100 assays

Microplate procedure: 500 assays

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Introduction

The Micro BCA Protein Assay Kit is a detergent-compatible bicinchoninic acid formulation for the colorimetric detection and quantitation of total protein. An adaptation of the BCA Protein Assay Kit, the Micro BCA Kit has been optimized for use with dilute protein samples (0.5-20 µg/ml). The unique, patented method utilizes bicinchoninic acid (BCA) as the detection reagent for Cu+1, which is formed when Cu+2 is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu+1). This water-soluble complex exhibits a strong absorbance at 562 nm that is linear with increasing protein concentrations.

The macromolecular structure of protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation is caused by more than the mere sum of individual color-producing functional groups.

The Micro BCA Protein Assay Kit uses concentrated reagents and a protocol that utilizes an extended incubation time at an elevated temperature (60°C, Test Tube Procedure only). The result is an extremely sensitive colorimetric protein assay in a test tube or microplate assay format.

Protocol

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin (BSA) Standard ampule into several clean vials, preferably using a diluent that is similar to the sample buffer. Reconstitute and dilute the lyophilized Albumin Standard Ampules with 1ml of diluent buffer (0.9% NaCl or PBS was recommended).

Table1. Preparation of Diluted Albumin (BSA) Standards

Vial	Volume of Diluents	Volume and Source of BSA	Final BSA Concentration
	7.84 ml	160 µl of 10 mg/ml dilution	200 µg/ml
A	8.0 ml	2.0 ml of 200 µg/ml dilution	40 µg/ml
B	4.0 ml	4.0 ml of A dilution	20 µg/ml
C	4.0 ml	4.0 ml of B dilution	10 µg/ml
D	4.0 ml	4.0 ml of C dilution	5 µg/ml
E	4.0 ml	4.0 ml of D dilution	2.5 µg/ml
F	4.8 ml	3.2 ml of E dilution	1 µg/ml
G	4.0 ml	4.0 ml of F dilution	0.5 µg/ml
H	8.0 ml	0	0 µg/ml =Blank

B. Preparation of the Micro BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   (# quantity of standard wells+ # quantity of unknown sample wells) × (# replicates) × (volume of WR per sample well) = total volume of WR required

   Example: For the standard Test Tube Procedure, each sample needs 3 dilutions and 2 replicates:

   (8 standard wells + 3 sample wells) × (2 replicates) × (1 ml) = 22 ml WR required (round up to 25 ml)

   Note: 1 ml of the WR is required for each sample in the Test Tube Procedure, while only 150 µl of WR is required for each sample in the Microplate Procedure.

2. Prepare WR by mixing 25 parts of Micro BCA Reagent **MA** and 24 parts Reagent **MB** with 1 part of Reagent **MC** (25:24:1, Reagent MA:MB:MC). i.e., mix 5 ml of Reagent **MA** and 4.8 ml Reagent **MB** with 0.2ml of Reagent **MC**.

Note: When Reagent **MC** is initially added to Reagents **MA** and **MB**, turbidity occurs that quickly disappears upon mixing to yield a clear-green solution.

Prepare sufficient volume of WR based on the quantity of samples to be assayed.

The WR is stable for one day when stored in a closed container at room temperature (RT). It is not necessary to protect the solution from light.

Assay Procedure

- **Test Tube Procedure (linear working range of 0.5-20 µg/ml)**

1. Pipette 1.0 ml of each standard and unknown sample replicate into appropriately labeled test tubes.
2. Add 1.0 ml of the WR to each tube and mix well.
3. Cover tubes and incubate at 60°C in a water bath for 1 hour.
4. Cool all tubes to room temperature (RT).
5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.

Note: Color development continues even after cooling to RT. However, the rate of development at RT is sufficient low that no significant error is introduced if all absorbance measurements are made within a 10 minute period.

6. Subtract the average 562 nm absorbance reading of the Blank standard replicates from the 562 nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm reading for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

- **Microplate Procedure (linear working range of 2-40 µg/ml)**

1. Pipette 150 µl of each standard or unknown sample replicate into a microplate well.
2. Add 150 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate using Sealing Tape for 96-Well Plates and incubate at 37°C for 2 hours.

Note: Limit incubations of microplate to less than or equal to 37°C, otherwise high background and aberrant color development may result. Most polystyrene assay plates deform, leach, and become cloudy at 60°C.

4. Cool plate to room temperature (RT).
5. Measure the absorbance at or near 562 nm on a plate reader.
6. Subtract the average 562 nm absorbance reading of the Blank standard replicates from the 562 nm reading of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm reading for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Note: If using curve-fitting software, use a best-fit polynomial equation rather than a linear equation for the standard curve. If plotting results by hand, a point-to-point fit is preferable to a linear fit to the standard points.